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Impact of Polyethylene Terephthalate Microplastics on Aerobic Granular Sludge Structure and EPS Composition in Wastewater Treatment

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Abstract: Aerobic granular sludge (AGS) is a promising technology for wastewater treatment. Granules have a compact microbial structure and a high potential for pollutant removal. Despite its advantages, the impact of microplastics (MPs) on AGS remains poorly understood, posing a potential risk to the stability and efficiency of biological wastewater treatment processes. This study investigates the effects of polyethylene terephthalate (PET) MPs on AGS structure and extracellular polymeric substance (EPS) composition, providing new insights into the interaction between MPs and AGS. Four granular sequencing batch reactors (GSBRs) were operated with varying concentrations of PET MPs in the influent wastewater (0, 1, 10, 50 mg/L). Key findings include MP-induced changes in granule size distribution, with an increase in smaller granules (<90 µm) observed in reactors exposed to PET MPs. EPS concentrations (51–77 mg/L) exhibited significant differences among reactors, with notable shifts in protein (PN) and polysaccharide (PS) fractions. A higher PET MP dose resulted in an increased PN/PS ratio (from 1.96 to 5.40) and elevated hydrophobicity of AGS. These changes suggest that MPs can alter AGS structure and EPS composition, potentially affecting granule stability and treatment performance. This study provides novel evidence on the disruptive effects of MPs in wastewater treatment systems, emphasizing the need to address MP pollution in the context of biological treatment processes. The results contribute to a deeper understanding of the interactions between MP and AGS and form the basis for strategies to mitigate their adverse effects.

Keywords: PET; micropollutants; biomass; proteins; polysaccharides; biological wastewater treatment

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1. Introduction

Emerging contaminants are prevalent across various ecosystems and are frequently detected in wastewater. A significant number of these contaminants successfully infiltrate ecosystems, particularly aquatic environments, via wastewater treatment plants (WWTPs) [1,2]. As a result, recent research has increasingly been devoted to studying the behavior and movement of emerging pollutants in wastewater and their impact on wastewater treatment processes, with a focus on biological treatment systems [2–5].

WWTPs collect wastewater from private household, industry, and commercial, as well as urban, runoff, including rainwater. Increasing population density, social habits, and the use of personal care products contribute to the heightened influx of microplastics (MPs)

into WWTPs [6]. During wastewater treatment, a notable portion of MPs (ranging between 10% and 55% of the total MPs entering with influent wastewater to WWTPs) is retained in the sewage sludge in the biological part of the WWTP. This retention illustrates the continued exposure of the biomass to the risks associated with these emerging pollutants [7]. Mahon et al. (2017) reported that in Ireland, up to 98% of MPs entering WWTPs were effectively removed from the effluent by retention in sewage sludge, with concentrations ranging from 4196 to 15,385 particles per kilogram in dewatered sludge samples [8]. Wang et al. (2023) reported that high concentrations of MPs negatively impacted the denitrification in activated sludge, leading to nitrate accumulation. In addition, MPs were found to reduce the total amount of extracellular polymeric substances (EPSs) and at the same time change EPS composition. This change was also found in the relative abundance of nitrogen-functional microorganisms and functional enzyme genes [9].

Aerobic granular sludge (AGS) stands out as a highly promising technology for biological wastewater treatment [10]. In comparison with conventional activated sludge systems, AGS offers numerous advantages, including a compact microbial structure, superior settleability, biological resilience to elevated organic loads and toxicity, and high potential for simultaneous nutrient removal [11]. Recently, research has increasingly focused on the interaction between emerging pollutants in wastewater and the performance of AGS [4,5]. Guo et al. (2024) reported that the presence of MPs can significantly influence the performance and structural properties of AGS, particularly at higher concentrations (100 mg/L). High MP concentrations negatively impacted biomass growth and settling performance. This was attributed to the induction of filamentous bacteria growth on the surface of AGS, which weakened the granule structure and disturbed the stability of the reactor [12].

The presence of EPSs significantly influences both the defense mechanisms and the transformation of micropollutants in biomass. The abundance of hydroxyl, carboxyl, sulfhydryl, and phosphate amine groups in EPSs contributes significantly to the sorption, transport, and alteration of micropollutants. Consequently, the interaction between EPSs and these pollutants affects their overall removal efficiency [13,14]. Numerous studies have shown that EPSs form a protective shield for cells against unfavorable environmental conditions, such as exposure to toxic substances [15]. This can lead to the formation of complexes between EPSs and pollutants through hydrophobic interactions, hydrogen bonding, or electrostatic interactions, which significantly affects the removal and migration of these pollutants in biological WWTPs [16]. Wang et al. (2022) explored the impacts of polystyrene (PS) MPs on AGS systems and demonstrated a particle size-dependent toxicity. Increasing PS MP sizes (0.5 μm to 150 μm) resulted in progressively greater inhibition of methane production (6.7–16.2%) and organic carbon degradation. Larger MPs caused more severe damage to the integrity of the sludge and microbial viability, while smaller MPs promoted EPS production, especially of humic substances, and partially attenuated toxicity. Conversely, larger MPs inhibited EPS secretion and exacerbated oxidative stress and structural damage to the sludge matrix [17]. The role of EPSs in the removal of micropollutants from wastewater has become a focus for many researchers [18,19].

Despite existing research on the influence of polyethylene terephthalate (PET) MPs on AGS, the specific interactions between MP and EPS fractions are still insufficiently characterized. Current studies have not clarified which EPS components are most susceptible to MP exposure, resulting in a limited understanding of the mechanisms by which these contaminants affect the structural integrity and functionality of AGS.

The present study aims to evaluate the impact of PET MPs, which constitute 22.0–29.9% of MPs detected in influent wastewater entering WWTPs [20], on the granulometry of AGS and the composition and abundance of EPSs. By addressing these interactions,

the research seeks to bridge a critical gap in understanding the effects of MPs on biological treatment processes.

This study provides novel insights into the specific effects of PET MPs on distinct EPS fractions, exposing their role in maintaining AGS stability and performance. These findings contribute to a more comprehensive understanding of the interactions between MPs and AGS and provide a basis for refining wastewater treatment strategies, thereby advancing the field of environmental biotechnology.

2. Materials and Methods

2.1. Reactor Setup

The AGS for reactor inoculation was obtained from amunicipal WWTP in Lubawa (53°30′19.84″ N, 19°43′53.15″ E). The biomass was cultivated in a granular sequencing batch reactor (GSBR) with an inner diameter of 10 cm, an operating height of 50 cm, and a working volume of 3.0 L. In the GSBR, the exchange ratio was 60%/cycle. Air bubbles (superficial air velocity of 0.8 cm/s) were introduced via diffusers at the bottom of the reactors to ensure oxygen supply and complete mixing. The GSBRs were operated at room temperature in cycles of 8 h, consisting of filling (5 min), the anoxic phase (60 min), the aeration phase (400 min), settling (10 min), and withdrawal (5 min). Peristaltic pumps were used to fill and withdraw wastewater at controlled flow rates [21]. The reactors were fed with synthetic wastewater [22] containing 800 mg COD/L (in the form of sodium acetate), 60 mg N-NH_4/L (NH_4Cl), 8 mg P-PO_4/L (Na_2HPO_4), 10.1 mg NaCl/L, 4.7 mg KCl/L, 243.3 mg $NaHCO_3$/L, 162.2 mg Na_2CO_3/L, 4.7 mg $CaCl_2$/L, 16.7 mg $MgSO_4$/L, and 0.2 mg/L of trace element solution ($FeCl_3 \cdot 6H_2O$, $ZnSO_4$, $MnSO_4 \cdot H_2O$, and $CuSO_4$).

During the experiments, wastewater containing PET MPs in concentrations of 1, 10, and 50 mg/L were fed to the GSBRs in R2, R3, and R4, respectively. Wastewater without PET MPs was fed to GSBR R1 (control). PET MPs (ultra-high molecular weight, powder, density 0.94 g/cm^3) with a particle size $\leq$ 300 µm were purchased from Goodfellow Cambridge Limited (London, UK). Before being introduced into the reactors, PET MPs were first added to barrels containing untreated wastewater. The mixture was homogenized using mechanical stirrers to ensure uniform distribution of the particles. The homogenized solution was then dosed into the GSBRs, ensuring consistent dispersion of MPs throughout the experimental process. COD, total nitrogen (TN), nitrogen ammonia (N-NH_4), and total phosphorus (TP) were measured according to APHA [23] in the influent and effluent from the GSBRs.

2.2. Biomass Structure

A wet sieving technique using the Retsch AS200 sieving device analyzed the particle size of AGS. Sieves of the following sizes were used: 90, 125, 250, 355, 500, and 710 µm. For sieving, 1000 mL of AGS was taken from each GSBR at the end of the experiment. The tap water (12 °C) used for sieving was administered through a spray nozzle above the top sieve. The water and biomass fraction < 90 µm left the sieve stack through the collector outlet. The sieving time for this experiment was 15 min, with a vibration amplitude of 1.5 mm [24].

2.3. Determination of EPSs

During stable operation of the GSBRs, three biomass samples were taken from each reactor after 20, 40, and 60 days of the experiment (the average value of two replicates is given in the results). Soluble EPSs (SOL-EPS), loosely bound EPSs (LB-EPS), and tightly bound EPSs (TB-EPS) were isolated from these samples. SOL-EPS remained in the supernatant after centrifugation for 15 min at 12,000× g and 4 °C. The resulting pellet was

resuspended in PBS buffer to reach the original volume. After vortexing for 5 min, another centrifugation (12,000× *g*, 4 °C, 15 min) detected LB-EPS in the supernatant. The pellet was resuspended in PBS buffer at its original volume. Subsequently, a cation exchange resin was used to extract TB-EPS according to the method described in Rusanowska et al. [25]. The concentrations of proteins (PNs) and polysaccharides (PSs) were determined by Frølund et al. [26] using the Lowry method with bovine serum albumin as a standard and the Anthrone method with a glucose standard curve, respectively.

2.4. Statistical Analysis

One-way ANOVA was applied to compare multiple groups of samples, followed by the HSD Tukey test in the Statistica 13.0 program (StatSoft, Tulsa, OK, USA). For correlation, the Pearson coefficient was used. The significance level (*p*-value) in this study was 0.05.

3. Results and Discussion

3.1. Pollutant Removal Efficiency in GSBRs

The analyses were carried out during stable reactor operation after 20, 40, and 60 days of the experiment. The pollutant removal efficiencies are summarized in Table 1. The average COD removal efficiency in the reactors ranged from 84.4% to 86.4%, indicating that the MPs did not have a significant effect on the removal of organic compounds. These results are consistent with those of Guo et al. (2024), who found that low and medium concentrations of MPs (10 and 50 mg/L) had no significant effect on COD removal in AGS systems. However, their study also highlighted that higher MP concentrations (100 mg/L) significantly reduced COD removal efficiency, primarily due to the obstruction of nutrient transport channels within the granular structure of AGS [12].

Table 1. Average pollutant removal efficiency (%) at sampling points (n = 3).

	R1	R2	R3	R4
COD	85.5 ± 4.7%	84.4% ± 5.3%	85.1% ± 7.4%	86.4% ± 7.5%
TN	98.0% ± 3.9%	94.3% ± 3.1%	95.0% ± 3.4%	96.9% ± 4.4%
TP	64.4% ± 4.2%	58.5% ± 5.4%	64.6% ± 4.4%	60.03% ± 6.2%

The average TN and TP removal efficiencies were 95.0–98.0% and 58.5–64.6%, respectively, demonstrating a lack of significant correlation between the dose of MPs introduced into the GSBR and the overall performance of wastewater treatment.

3.2. Biomass Characteristics of AGS

The presence of MPs was shown to influence the structure of AGS (Figure 1). The predominant granule sizes in the GSBRs were within the ranges of 125–250 µm (33–42%), 250–355 µm (25–37%), and 90–125 µm (5–19%). It was shown that the MP dosage significantly increased the proportion of granules with diameters < 90 µm ($r = 0.97$, $p < 0.05$). In R1-R3, the proportion of these granules was between 5 and 6% but increased notably to 10% in R4. This increase could be due to the long-term presence of high MP concentrations in the wastewater, which led to the breakage of the granules [27]. The proportion of granules with a size of 90–125 µm decreased with MP dosage from 19% in R1 to 5% in R4. The share of the granules with sizes of 125–250 µm and 250–355 µm increased progressively with MP dosage, indicating that MPs were incorporated into the granule structure, as previously reported by other researchers [28,29].

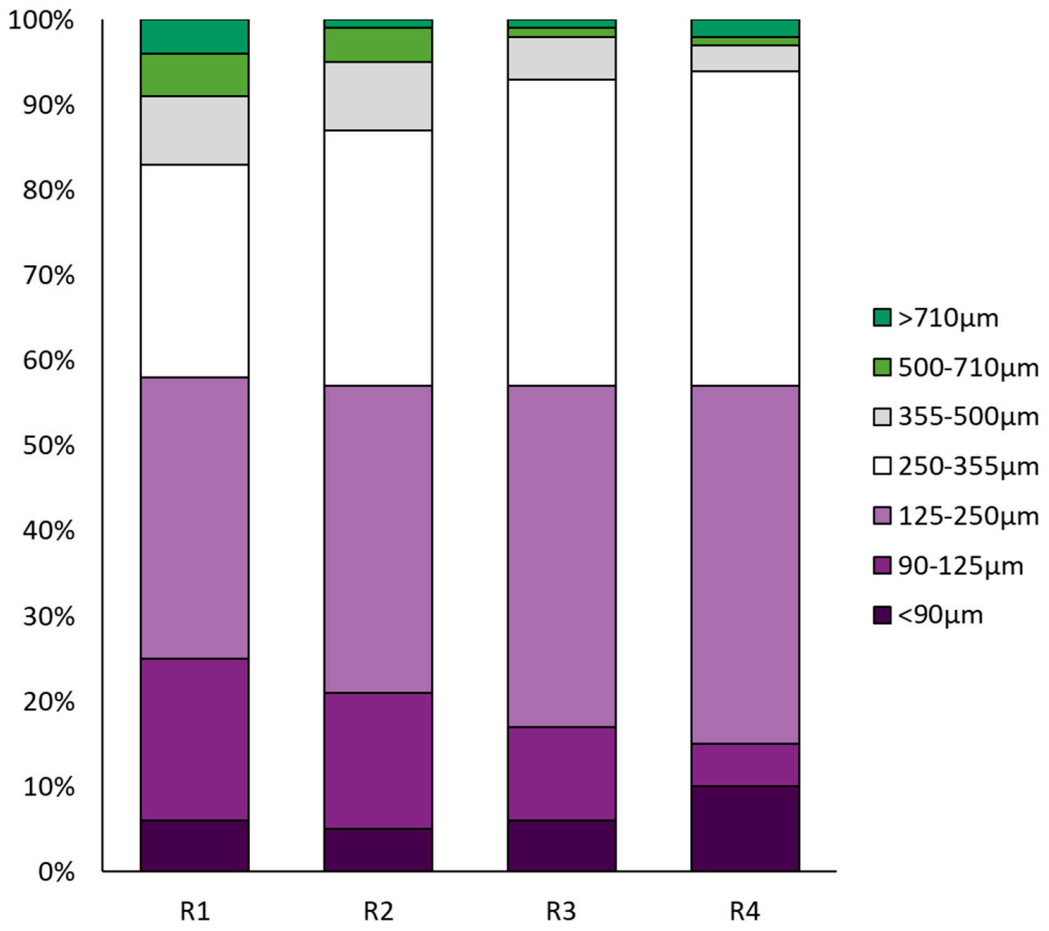


Figure 1. The distribution of granule particle sizes in the reactors.

Granules with a size of 355–500 µm accounted for 8% of the biomass in R1 and R2, while higher MP dosages decreased their proportion in the biomass to 5% and 3% in R3 and R4, respectively. Granules with a size of 500–710 µm represented 1–5% of the total biomass, and their proportion in the biomass decreased with the increasing dose of MPs in the wastewater. A negative correlation was found between the abundance of these granules and those with a size of 250–355 µm ($r = -0.98$) and 125–250 µm ($r = -0.97$). This negative correlation indicates that larger granules broke down into smaller sizes with increasing MP exposure. The disintegration of the granules can be caused by the collision of MPs with the AGS. These interactions can lead to mechanical stress and damage to the AGS and affect its structural integrity and stability. For granules > 710 µm in size, their proportion in biomass was between 1 and 4% and was not affected by the dose of MPs in the wastewater.

3.3. EPS Distribution in AGS

The effect of MP dosage on the total EPS concentration and its specific fractions was observed (Figure 2). In the reactors, total EPS concentrations, calculated as the sum of PNs and PSs, ranged between 51 and 78 mg/L. When the EPS concentration decreased, this may indicate a reduced ability of the biomass to granulate [30]. Conversely, research has shown that increasing EPS levels, with high EPS concentrations, promotes the reduction in the sludge volume index [31]. In R1 and R2, the concentrations remained constant between 71 and 77 mg/L throughout the experiment. However, in R3 and R4, the total EPS concentrations declined on day 40 compared to day 20, before increasing again towards the end of the experiment, reaching 62 ± 3 mg/L and 64 ± 4 mg/L, respectively.

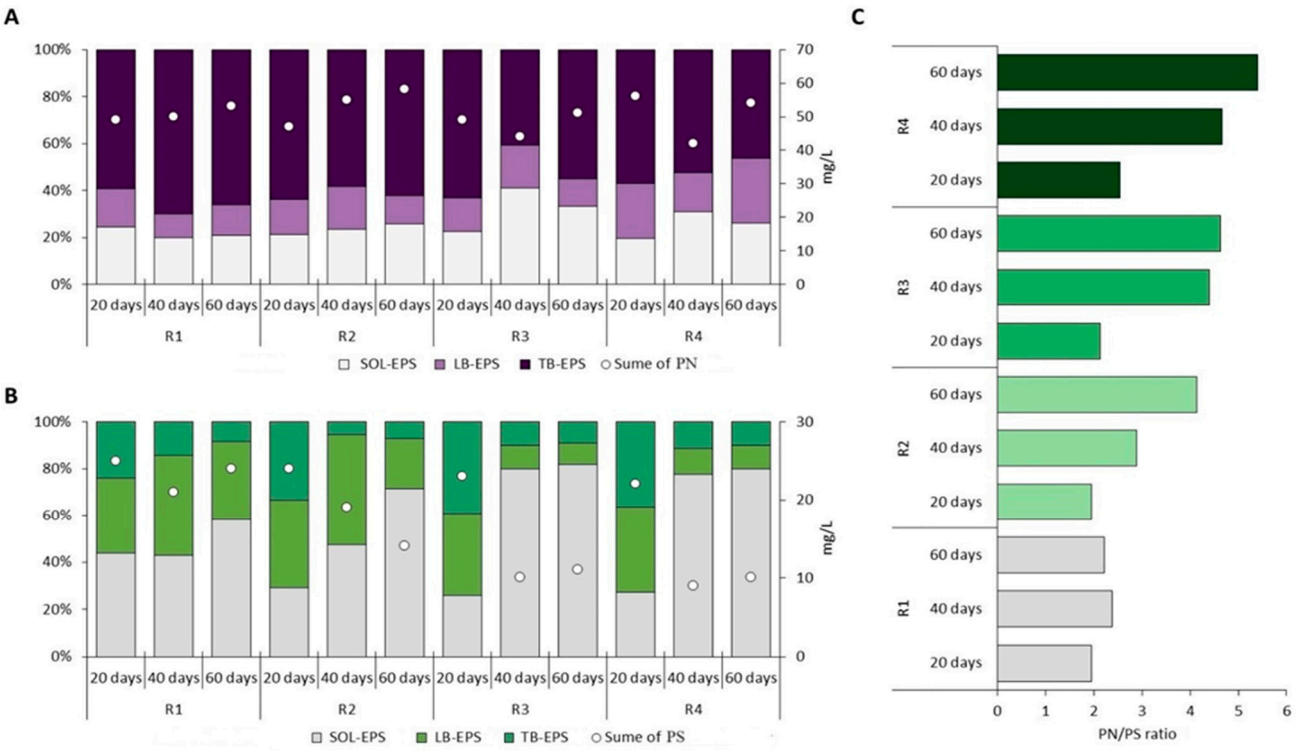


Figure 2. The PN (**A**) and PS (**B**) contents, and the PN/PS ratio (**C**) of the EPS fractions in AGS during the experiment.

A study conducted by Zhang et al. [32] demonstrated that high doses of PET MPs significantly reduced EPS levels. This reduction was attributed to the potential of MPs to induce microbial mortality, thereby suppressing EPS secretion. Notably, the type of MP appears to influence the structure and concentration of EPSs differently. For example, previous studies on the effects of PET MPs indicated that the total EPS concentration increased with the duration of the experiment and the dose of MPs in the wastewater [28].

The research results revealed a significant correlation between PN concentration and total EPS concentration ($r = 0.88$, $p < 0.05$, Figure 3). This correlation is explained by the fact that PNs accounted for 66% to 84% of the total EPS, establishing PNs as the primary component of EPSs. It is hypothesized that the positively charged amino groups in PNs neutralize the negative charges of PSs, uronic acid, carboxylic acid in DNA, or phosphate groups. This property of PNs effectively modifies the surface properties of the sludge, promoting cohesion between aggregates and maintaining the dense, stable structure of AGS [33–35].

The PN/PS ratio increased proportionally with the increase in MP dosage in the reactors (Figure 2). In R1, the PN/PS ratio remained relatively constant throughout the experiment (1.96–2.38). However, in the reactors supplemented with MPs, the PN/PS ratio consistently increased and correlated with the MP dose in the wastewater ($r = 0.60$, $p < 0.05$). By the end of the experiment, the PN/PS ratio was 4.14, 4.63, and 5.40 for R2, R3, and R4, respectively. The high PN/PS ratio, which may contribute to increased AGS hydrophobicity, supports the protection of microorganisms and facilitates communication under challenging conditions [36]. These findings are consistent with previous research, which demonstrated that microorganisms increase EPS secretion, especially PNs, to adapt to challenging external conditions [37].

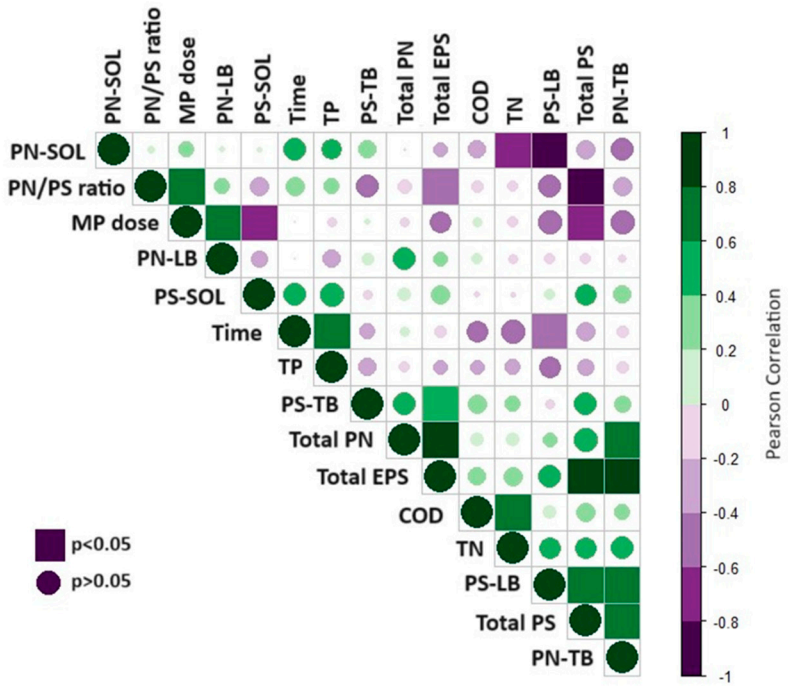


Figure 3. Correlation between concentration of EPS compounds and physicochemical parameters of wastewater.

During the experiments, the total concentration of PNs in EPSs (PN-EPS) within AGS ranged from 42 to 58 mg/L. There were clear differences between the reactors: in R1 and R2, the amount of PN concentration increased steadily over time while in R3 and R4, the concentration of PN-EPS decreased on the 40th day of the experiment and increased again on the 60th day.

The PN concentration in the TB-EPS fraction varied between 18 and 36 mg/L, accounting for 37% to 73% of the total PN-EPS fraction. A significant correlation ($r = 0.72$, $p < 0.05$) was observed between changes in the TB-EPS concentration and the total PN concentration, indicating that PNs had the greatest influence on the total TB-EPS content. Consequently, the PN concentration in R1 and R2 increased steadily over time (from 29 to 36 mg/L). In contrast, in R3 and R4, the PN concentration was lower than the initial levels observed at the start of the experiment. Previous studies demonstrated that proteins are predominantly located in the EPS fraction as TB-EPS (97%), whereas humic acids are distributed between both TB-EPS and SB-EPS fractions. This observation is consistent with the results of the present study, further highlighting the role of proteins in the TB-EPS fraction [38].

As the primary component of TB-EPS, PNs play a more critical role in biomass aggregation than PSs [39]. This suggests that MPs may interfere with the granulation process by disrupting the structural and functional balance of TB-EPS, potentially challenging the stability of the sludge.

PNs accounted for 10% to 31% of the LB-EPS fraction. A significant correlation ($r = 0.61$, $p < 0.05$) was observed between LB-EPS content and MP dosage. Notably, R4 exhibited substantial variability, with the LB-EPS fraction increasing significantly from 27% on day 20 to 31% on day 60. The accumulation of large quantities of EPSs, especially in the form of LB-EPS, was accompanied by a deterioration of the adhesive properties and a weakened aggregate structure. This destabilization facilitated the erosion of slime flocs and the detachment of cells, which may have been exacerbated by turbulence [40].

No significant correlations were found for PNs in the SOL-EPS fraction concerning MP dosage or duration of the experiment. However, R3 showed a notable increase in PN concentration in the SOL-EPS fraction, rising from 11 to 17 mg/L, while R4 maintained

a relatively constant range of 11–14 mg/L. In contrast to these results, studies by Wang et al. (2020) showed that nanoparticles significantly affect not only the protein content in EPSs but also the types and structures of proteins. For example, three-dimensional fluorescence spectroscopy showed a decrease in the content of tyrosine-like proteins in SOL-EPS after exposure to different nanoparticles [41]. A negative correlation was found between SOL-EPS and ammonium removal efficiency ($r = -0.61$, $p < 0.05$). This correlation can be attributed to the release of previously sorbed ammonium nitrogen from the SOL-EPS fraction into the liquid phase, possibly reducing the ammonium removal efficiency [42].

The PS concentration varied between the reactors and throughout the experiment. A significant negative correlation was found between total PS concentration and MP dosage ($r = -0.65$, $p < 0.05$). In R1, the PS concentration remained relatively stable and ranged between 21 and 25 mg/L. However, in the reactors exposed to MP-containing wastewater (R2, R3, and R4), the PS concentration decreased to less than 15 mg/L by the end of the experiment. This decrease in PS concentration is probably due to the stronger tendency of PS to adsorb to MPs compared to other EPS constituents [43].

The PS concentration in the TB-EPS fraction ranged from 5% to 39%. Throughout the experiment, the TB-EPS concentration in R1 remained stable at 5–6 mg/L. However, on day 20, the TB-EPS concentrations in the reactors supplemented with MPs were significantly higher (8–9 mg/L), suggesting increased polymer production in response to the presence of micropollutants in the environment [44]. In the following days, these values decreased sharply to 1–3 mg/L, suggesting that chronic exposure to MPs negatively affects TB-EPS secretion. According to Rusanowska et al. [25], each EPS fraction in AGS corresponds to different microenvironmental conditions, with TB-EPS playing a crucial role in maintaining more stable conditions that support the growth of sensitive microbial species. This highlights the potential of MPs to disrupt these protective mechanisms and compromise the resilience of the granular structure.

The presence of MPs in wastewater affected the concentration of PSs in the LB-EPS fraction of the biomass. In R1, the concentration remained relatively stable throughout the experiment (8–9 mg/L). In R2, it remained constant until the 40th day (9 mg/L) but decreased significantly until the 60th day. In R3 and R4, which were operated at the highest MP doses, the concentration dropped drastically to below 1 mg/L by day 40.

The decrease in LB-EPS content can be attributed to collisions between MPs and this EPS fraction, which is probably due to the lower shear resistance of LB-EPS [45]. In addition, the significantly lower MP dose in R2 could explain the delayed observation of this interaction compared to R3 and R4. Another possible reason for the decrease in LB-EPS and TB-EPS concentrations could be the sorption of PS fractions from the AGS core by MPs present in the water column. This phenomenon could be due to interactions between the negatively charged PSs and the surface properties of the PET MPs [46,47].

The proportion of PSs in the SOL-EPS fraction ranged from 26% to 82%. A significant negative correlation was found between SOL-EPS concentration and MP dose ($r = -0.66$, $p < 0.05$). In R1, the proportion of PSs in SOL-EPS remained relatively stable and ranged between 44% and 58% throughout the experiment. However, in the reactors exposed to MPs, the proportion of PSs in the SOL-EPS fraction increased. This increase is likely a consequence of the decrease in PS concentrations in other EPS fractions, indicating that the PSs that originally bound to the biomass were transferred to the surrounding liquid phase [32,48].

3.4. Comparison of Results with Existing Research

In this section, we compare the findings of our study with those from relevant research, as illustrated in Figure 4 [28,49–60]. This comparative analysis underscores the similarities and differences in the effects of various MPs on EPS composition and quantity.

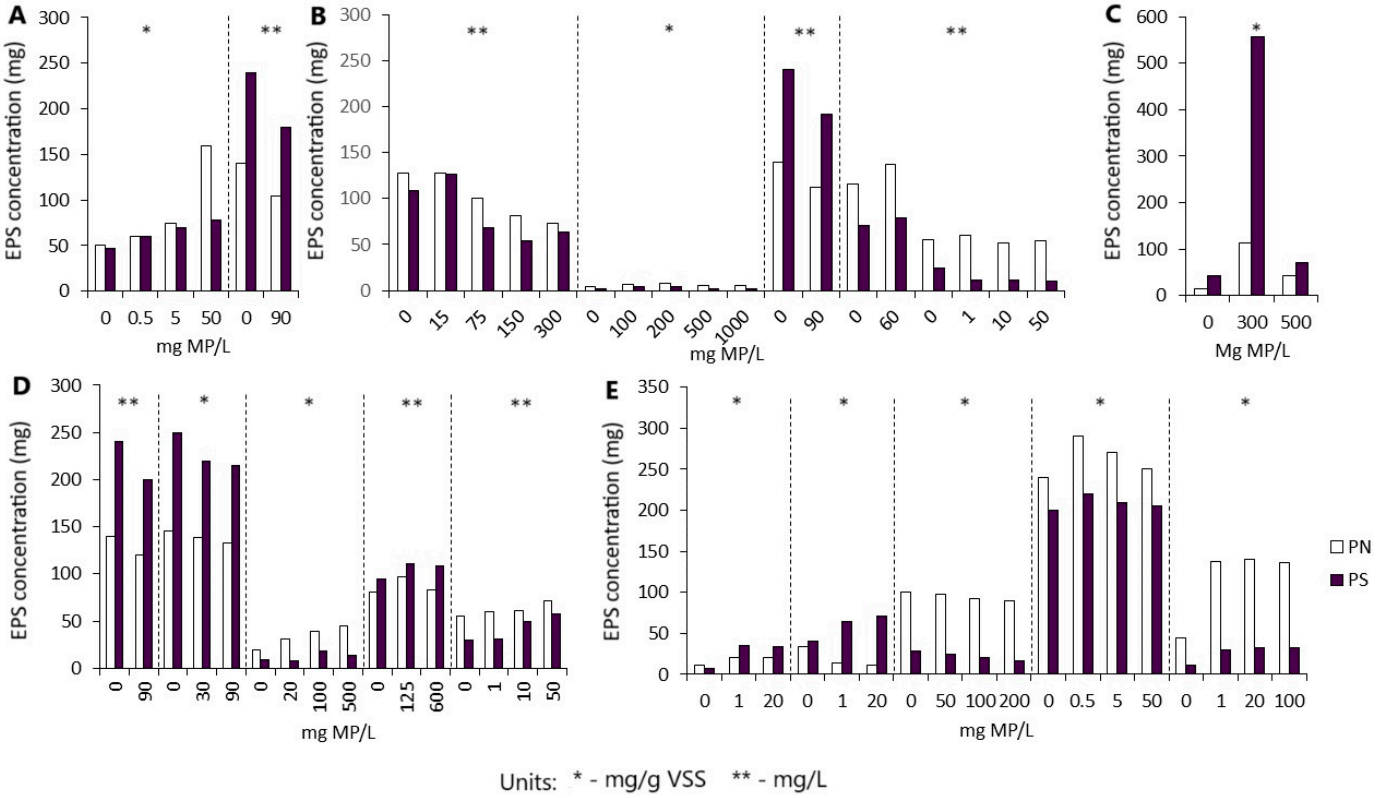


Figure 4. Effect of MP type and dose on EPS concentration in AGS ((**A**): PVC; (**B**): PET; (**C**): PP; (**D**): PE; (**E**): PS) [28,49–60]. (Dashed line represents separation between different studies).

The data presented in Figure 4 show that EPS concentration varies considerably depending on the type and dose of MPs. For PVC, the highest EPS concentration of 159 mg/L was observed in the PN fraction at a dose of 50 mg/L, while the PS fraction reached a maximum of 290 mg/L at a dose of 0.5 mg/L. PET showed different trends, with the PN fraction reaching a value of 137.48 mg/L at 60 mg/L, and the PS fraction peaking at 192 mg/L at 90 mg/L. PE and PP also showed dose-dependent variations in EPSs, with PE reaching a maximum of 215 mg/L at 90 mg/L in the PS fraction, and PP reaching a peak value of 557 mg/L at 300 mg/L. These results suggest that both the type and dose of MPs play a crucial role in influencing EPS production, with certain combinations either increasing or decreasing EPS concentrations.

Our results are consistent with other studies and show that PN concentration in EPSs decreased in all studies when the highest MP dose was compared with the control sample. Similarly, PS concentration in EPSs was lowest at the highest MP doses compared with the controls. For example, Zhang et al. (2020) reported that a dose of 15 mg MP/L increased EPS production, while higher doses generally resulted in decreased production [32].

When studying the effects of MPs, it is crucial to consider factors such as size, shape, and chemical additives in each type of MP. In most studies, MP concentrations are reported as mass (mg/L) rather than number of particles. This distinction is important, as the number of particles can vary significantly depending on size—smaller MPs result in a higher particle count compared to larger MPs at the same mass concentration. Gong et al. (2023) observed the effect of MPs of different sizes (0.065 μm, 0.5 μm, and 5 μm) on algal

biomass recovery and EPS composition. After 14 days, biofilms exposed to 0.065 µm MPs produced 16.61 ± 3.10 mg EPS/g, which decreased to 13.24 ± 2.12 mg/g by day 21. Conversely, biofilms exposed to 5 µm MPs initially produced 6.03 ± 0.07 mg EPS/g and peaked at 21.10 ± 0.91 mg/g by day 21. These results suggest that smaller MPs (e.g., 0.065 µm) have a more rapid effect on EPS production, while larger MPs (e.g., 5 µm) have a stronger long-term effect [61].

Understanding the interactions between MPs and EPSs is crucial, as these interactions are influenced by the different physical and chemical properties of MPs, including surface charge, hydrophobicity, and chemical additives. For example, MPs with higher surface hydrophobicity may bind more effectively with EPS components, potentially disrupting the balance between tightly bound and loosely bound EPS fractions [14,62].

Research has shown that the hydrophobic nature of certain MPs, such as PSs, leads to stronger interactions with hydrophobic organic pollutants due to π–π and hydrophobic interactions. This suggests that MPs with different degrees of hydrophobicity, such as PVC, PET, PE, and PP, may interact differently with EPS constituents, which may influence microbial aggregation and biofilm formation [63].

The presence of chemical additives in MPs can have significant effects. For example, plasticizers used in PVC are known to leach and disrupt microbial communities by impairing endocrine systems or suppressing immune responses. This disruption can alter microbial activity and EPS secretion and affect the stability and function of biofilms [64,65].

In addition, the surface charge of MPs plays a role in their interaction with EPSs. Electrostatic interactions occur when MPs and pollutants have opposite charges, which influences the adsorption processes. The charge of MPs is influenced by factors such as pH and the point of zero charge, which can alter the interaction of MPs with EPSs and associated microbial communities [47].

This topic is inherently interdisciplinary, spanning the fields of environmental science, chemistry, and biology. Furthermore, many studies simply divide EPSs into the overall PN and PS fractions. However, our study highlights the importance of examining individual fractions within these categories, such as TB-EPS and LB-EPS, as these can change significantly under different conditions. The use of detailed EPS fractionation enables a deeper understanding of the mechanisms governing the interactions between MPs and microbial communities and offers valuable insights into these complex dynamics.

4. Conclusions

The results of the study show that the presence of MPs significantly influences the structure of AGS. The introduction of MPs leads to granule break up as evidenced by an increase in the proportion of smaller granules (<90 µm) and a decrease in granules with a size of 355–710 µm. Conversely, the abundance of granules with a diameter of 125–355 µm increased, indicating the inclusion or incorporation of MPs into the AGS structure. The total EPS concentration in the biomass varied as a function of MP dose. The increasing PN/PS ratio with increasing MP dose increased the hydrophobicity of the AGS. The presence of MPs primarily caused changes in PS concentration, especially its decrease within the AGS structure, and mainly led to a decrease in LB-EPS and TB-EPS fractions. These results highlight the complex interaction of MPs with the structure and composition of granular biomass, which could have significant implications for wastewater treatment.

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